Shell Microstructure and Observations on Internal Banding Patterns in the Bivalves *Yoldia thraciaeformis* Storer, 1838, and *Nuculana pernula* Müller, 1779 (Nuculanidae), from a Deep-Sea Environment[1]

by

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Abstract. Shell microstructure and internal banding patterns are described for deep-sea (895–1490 m) populations of *Yoldia thraciaeformis* and *Nuculana pernula* (Bivalvia: Nuculanidae) from the northwest Atlantic Ocean off Newfoundland. Both species possess homogeneous, aragonitic shell microstructure. The shell of *N. pernula* is comprised of two distinct shell layers whereas that of *Y. thraciaeformis* has one layer. Internal bands (growth increments) are prominent within the shells of both species. Shell microstructure is similar within and between band regions. Internal bands may be deposited annually in response to discrete seasonal or annual pulses of surface production to the deep sea.

INTRODUCTION

SHELL MICROSTRUCTURE and mineralogy in bivalves have been the focus of many paleoecological and biological examinations which have been useful in understanding phylogenetic relationships among bivalves and documenting periodic growth increments (banding patterns) within the shells of these mollusks (see LUTZ & RHOADS, 1980, for a review).

TAYLOR *et al.* (1969) examined the shell structure and mineralogy in 12 species within the Nuculanacea and found all shells to be entirely composed of aragonite. Furthermore, a homogeneous shell microstructure is characteristic among all species except *Yoldia eightsii*, which has a prismatic microstructure underlying the pallial myostracum. The existence of either one or two shell layers varies within and between genera. For example, *Y. lima-tula* and *Nuculana crassa* each possess two shell layers whereas the shells of *Y. eightsii, Y. myalis,* and *N. oblongoides* are comprised of a single layer (TAYLOR *et al.,* 1969). Shells of the nuculanaceans *Y. thraciaeformis* and *N. pernula* are here documented for the first time in terms of their mineralogy, microstructure, and shell layers.

Growth increments in bivalves are understood to be records of growth (LUTZ & RHOADS, 1980). The underlying cause of growth increment formation in bivalves has been variously attributed to both environmental and physiological processes (JONES, 1981). Examples from the literature dealing with shallow-water bivalves reveal changes in shell growth rate at a time when growth bands are deposited (MACDONALD & THOMAS, 1980; PETERSON *et al.,* 1983; SHAUL & GOODWIN, 1982; DEITH, 1985). In some cases associated changes in crystal size between band and interband shell material have been recorded (DEITH, 1985; LUTZ, 1976). The majority of growth increment studies on bivalves have been undertaken on populations inhabiting shallow-water habitats (<60 m) which are subjected to dramatic seasonal and annual environmental fluctuations. Previous shell studies of deep-sea bivalves (>500 m) have included growth rate investigations based

upon radioisotope analysis (TUREKIAN *et al.*, 1975, 1979). Internal banding patterns were documented in the deep-sea clam *Tindaria callistiformis* collected from depths of 3800 m, although shell microstructure was not reported (TUREKIAN *et al.*, 1975). In a study of molluscan micro-growth patterns, RHOADS & PANNELLA (1970) recorded indistinct banding patterns in specimens of several deep-sea (933–4970 m) bivalve species, including the proto-branch *Nucula cancellata*.

Shell microstructure and internal banding patterns were examined from specimens of *Yoldia thraciaeformis* and *Nuculana pernula* collected from a deep-sea environment (895–1490 m) off Newfoundland. In a previous growth and population structure study of these populations, HUTCHINGS & HAEDRICH (1984) postulated that internal growth increments (bands) present in the shells of these species were of the first-order type (annual), as defined by BARKER (1964).

Bands were analyzed in detail through scanning electron microscopy in order to document any variation in shell microstructure concomitant with band formation. The suspected annual periodicity of band deposition in these bivalves is discussed in terms of the physical conditions that exist in this deep-sea environment and their implications to influences upon biological cycles.

The basic terminology is that of CARTER (1980). Hereafter, shell architecture refers to the orientation of the largest units of shell microstructure with respect to shell form. Shell microstructure refers to the arrangement of the various basic structural units (*e.g.*, granules).

MATERIALS AND METHODS

Deep-water specimens of *Yoldia thraciaeformis* and *Nuculana pernula* were collected during cruises of the M/V *Gadus Atlantica* in Carson Canyon, on the southeastern edge of the Grand Banks in the northwest Atlantic (Table 1; see HUTCHINGS & HAEDRICH, 1984, for collection procedure). Material was fixed in 10% formalin at the time of collection and later transferred to 80% ethanol. The *Y. thraciaeformis* analyzed were 35–45 mm and the *N. pernula* were 20–25 mm in shell length.

Light Microscopy

Shell valves of *Yoldia thraciaeformis* were fractured radially (passing through the umbo) for viewing of internal banding. Vertical sections were photographed (×16 power) using a 16-mm Zeiss Luminar lens with bellows, a Leica R3MOT back, and fiber optic illumination.

Scanning Electron Microscopy (SEM)

Shells were washed in absolute ethanol, air-dried, and vertically fractured along a radial plane. The fragments were further broken into smaller pieces to fit on SEM stubs. Specimens were also fractured at an angle tangential to the shell surface to produce a horizontal fracture surface. Shell fragments were mounted on aluminum SEM stubs with silver conducting paint, sputter coated with gold, and then examined in a Hitachi S-570 SEM or a Cambridge MK2A Stereoscan.

Shell Internal Band Microstructure

Two vertical notches (*i.e.*, normal to the outer shell surface) were made in shell sections of *Yoldia thraciaeformis* to frame a segment of a band region for comparison by light microscopy and scanning electron microscopy. The notched piece of shell was mounted on an SEM stub and shell microstructure was compared within and between bands.

Shell Mineralogical Determinations

Periostracum was scraped from shells of both species in preparation for grinding. Through SEM analysis it was apparent that there was a single shell layer in *Yoldia thraciaeformis,* whereas there were two distinct shell layers in *Nuculana pernula.* The two shell layers of *N. pernula* were analyzed separately. This was accomplished by removing shell material from both inner and outer shell surfaces with a scalpel.

Shell material from single specimens was ground to a fine powder with mortar and pestle. Smear-slide preparations were made by making a slurry of powdered carbonate in a drop of methanol, and spreading this slurry in a thin layer on a glass slide for mounting in an X-ray diffraction instrument. Analyses were conducted using a Philips Co. X-ray diffraction unit and recorder, and a goniometer.

RESULTS

Shell Architecture, Microstructure, and Mineralogy

Yoldia thraciaeformis shells were 1–2 mm thick. The periostracum was relatively thick although absent from the eroded umbonal regions. *Nuculana pernula* possessed a thin and fragile shell (about 0.5 mm thick) with a thin periostracum which also was eroded at the umbones.

Nuculana pernula possessed two distinct shell layers, which were separated by the pallial myostracum (Figure 1a). Both layers had a homogeneous microstructure. In these vertical shell sections there was a distinct difference in the density of structural units (granules) between the two shell layers. The inner shell layer consisted of compactly arranged shell material (Figure 1b) in which it was difficult to identify individual structural units. Structural units in the outer shell layer (Figures 1c, d) consisted of irregularly arranged spherical granules, with a high degree of porosity. Granules (smallest discrete units) were 0.8 μm in diameter.

Figure 2 shows inner and outer shell layers in a plane horizontal to the outer shell surface. Granules were coalesced into a sheet-like arrangement in the inner shell layer (Figures 2a, b). They appeared to be "cemented"
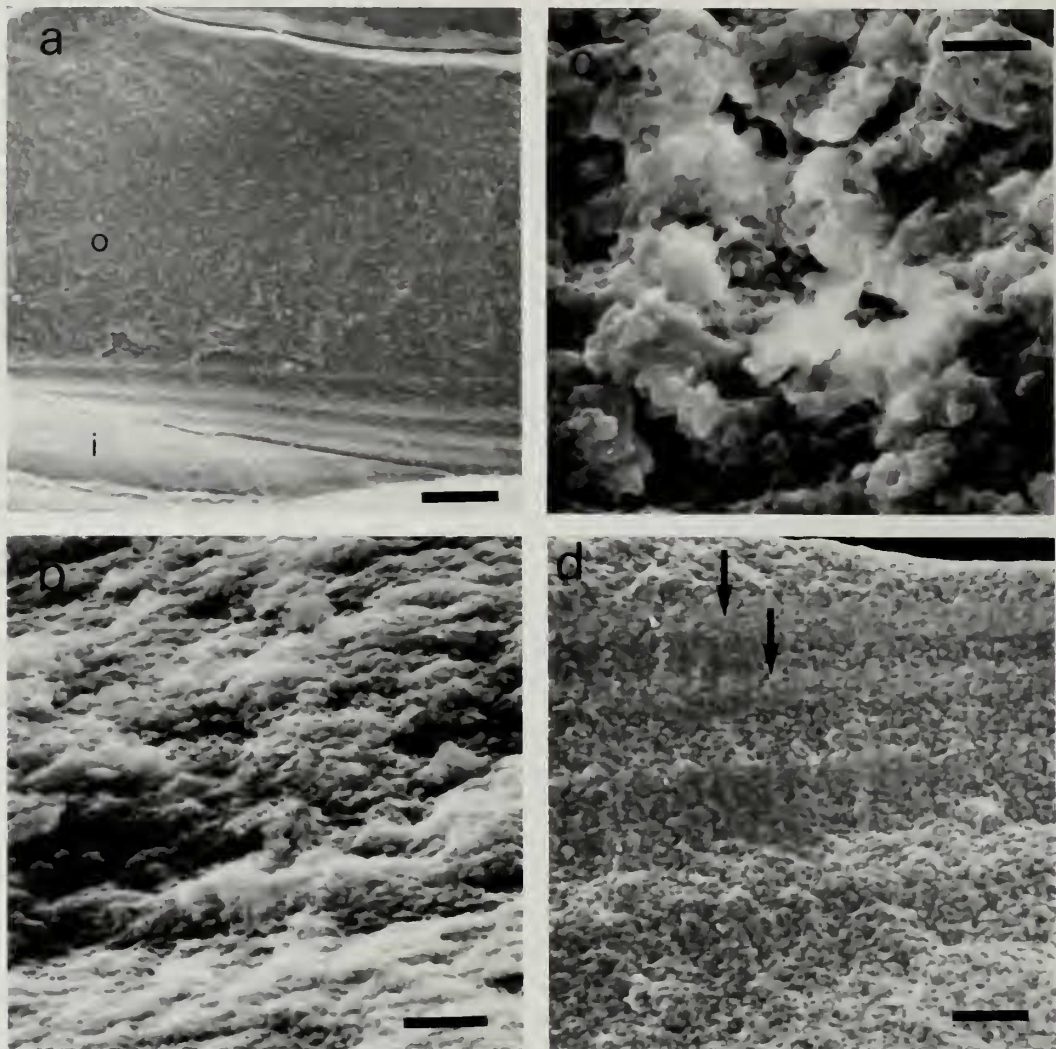
Figure 1

Radial, vertical shell sections of *Nuculana pernula*. a. Inner (i) and outer (o) shell layers (scale bar = 100 μm). b. Inner shell layer (scale bar = 2 μm). c. Outer shell layer (scale bar = 2 μm). d. Outer shell layer. Arrows point to internal bands (scale bar = 50 μm).

together by an amorphous deposit of calcium carbonate. Outer shell layer microstructure consisted of irregularly arranged granules 0.2–0.4 μm in greatest dimension (Figures 2c, d). Thus, granule sizes in the horizontal plane were roughly half of those in the vertical plane.

Yoldia thraciaeformis possessed a single, homogeneous shell layer. In radial, vertical section (Figures 3a, b, c) this consisted of densely packed, irregularly arranged granules. Shell microstructure in the horizontal plane (Figures 3d, e) was very similar to that seen in the vertical plane.

In summary, no pattern was evident in either species in the arrangement of granules within shell layers. There

was an apparent difference in density of granules between the two shell layers in *Nuculana pernula*. Shell calcium carbonate in both species was in the form of aragonite.

Internal Shell Banding Patterns

Internal banding patterns were readily apparent at low magnification (×16) in vertical shell sections of both species. The bands in *Yoldia thraciaeformis* were visible without magnification. Growth bands in *Y. thraciaeformis* were studied at the microscopic and microstructural levels (Figures 4a, b). Bands extended throughout the shell, running parallel to the inner shell surface, gradually becom-
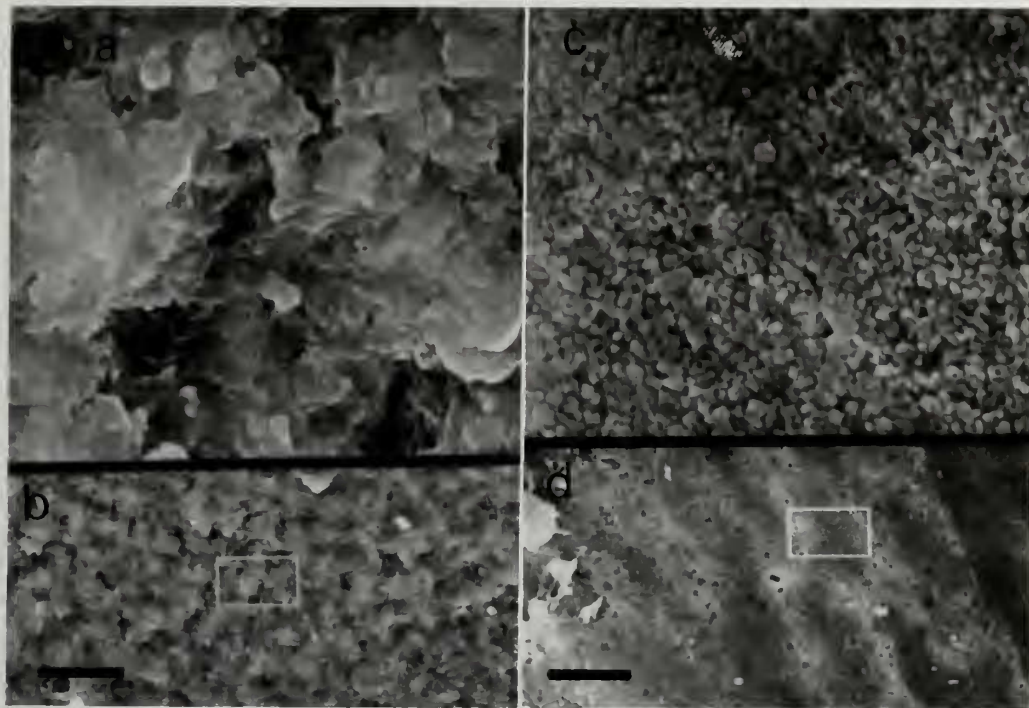
Figure 2

Horizontal plane views of *Nuculana pernula* shell. a. Inner shell layer magnification of inset region in "b" (scale bar = 1 μm in "a" and 10 μm in "b"). c. Outer shell layer magnification of inset regions in "d" (scale bar = 1 μm in "c" and 10 μm in "d").

ing deflected toward the outer shell surface (Figure 4a). In reflected light, individual bands (0.08–0.10 mm in width) appeared dark in contrast to the surrounding lighter colored interband shell surface. In transmitted light, the reverse was true. Shell microstructure appeared similar inside and outside a band as viewed at a band-interband border region (Figure 3a). Band spacing was not regular and interband distances were widest toward the umbo, becoming progressively narrower toward the ventral shell margin. Nevertheless, bands were markedly periodic and there were no unusually large gaps without bands.

The band delimited by the two notch marks in Figure 4a is shown as viewed under SEM and appears as a light "streak" in the center of Figure 4b. Figures 3a, b, and c show various magnifications of this band region. Grooves appearing in band regions appear to indicate lines of fracture.

Banding in *Nuculana pernula* was confined largely to the inner shell layer. The exception occurred at the inner shell-outer shell boundary where banding extended briefly into the the outer shell layer (Figures 1a, d). Bands were non-reflected and were oriented parallel to the shell surface. Bands were uniformly closely spaced and, in this thinner-shelled species, were narrower (about 13 μm) than in *Yoldia thraciaeformis*.

DISCUSSION

Both *Nuculana pernula* and *Yoldia thraciaeformis* possess homogeneous shell microstructures and aragonite mineralogy in conformity with other nuculanacean species studied to date.

Nuculana pernula possesses two distinct shell layers whereas *Yoldia thraciaeformis* develops only a single shell layer. Given the current state of knowledge of the calcification process in mollusks, it is difficult to speculate upon reasons for this variation in shell layer systems within and among genera in the Nuculanacea. However, this variation cannot be attributed simply to thin-shelled *vs.* thick-shelled bivalve life history strategies as both thin-shelled and thick-shelled species within the Nuculanacea can possess either one or two shell layers (TAYLOR *et al.*, 1969).

Both *Nuculana pernula* and *Yoldia thraciaeformis* possess internal banding patterns throughout their shells but on different scales. In the thin-shelled *N. pernula*, bands were thin (13 μm) and were largely confined to the inner shell layer. This banding pattern may be similar to that reported for other thin-shelled nuculanacean species by TAYLOR *et al.* (1969). Banding patterns within *Y. thraciaeformis* shells are similar to descriptions of the thick-shelled *N. crassa* and *N. oblongoides* by TAYLOR *et al.* (1969). These authors remark upon the similarity to first-
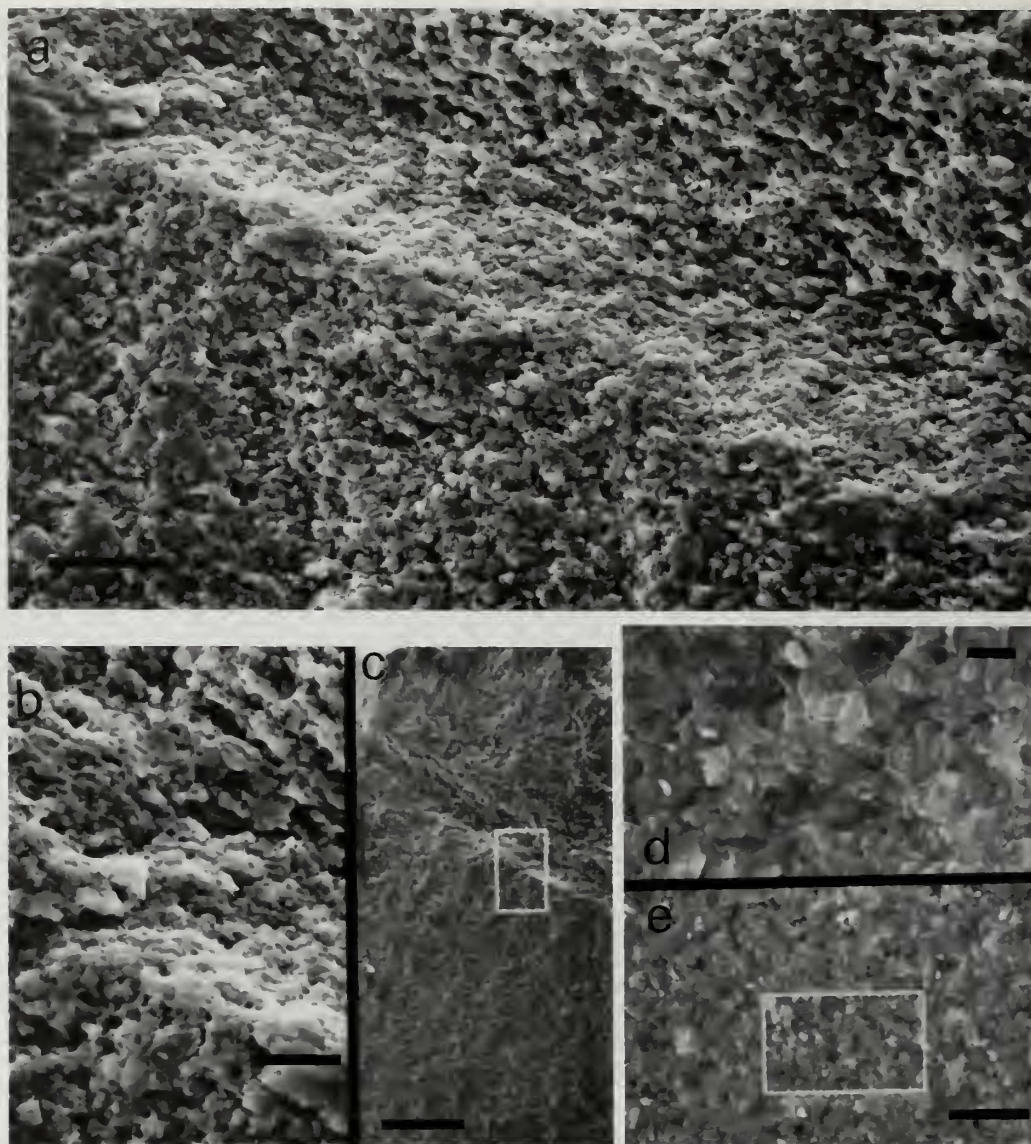
Figure 3

Radial, vertical shell sections of *Yoldia thraciaeformis*. a. Internal band region running diagonally across photograph from top left to bottom right (scale bar = 20 μm). b. Magnification of a region of an internal band identified by the inset in "c" (scale bar = 20 μm). c. Internal band region (scale bar = 100 μm). Horizontal plane views of *Y. thraciaeformis* shell. d. Magnification of inset region in "e" (scale bar = 1 μm in "d" and 10 μm in "e").

order banding patterns seen in *Arctica islandica,* an observation corroborated in the present study. The existence of fracture zones along growth bands in *Y. thraciaeformis* would seem to indicate a difference in density or structural strength between band and interband shell material even though differences in microstructure were not observed.

The fact that deep-water bivalves such as *Yoldia thraciaeformis* have internal banding patterns is not in itself surprising considering the variety of biological and environmental factors that may be responsible for growth increment formation in bivalves (see LUTZ & RHOADS, 1980, for a review). What is interesting is the remarkable definition or clarity of banding patterns seen in *Y. thraciaeformis* considering the fact that RHOADS & PANELLA (1970) observed indistinct banding patterns in the deep-sea bivalves they examined. They observed banding patterns that consisted of uniformly spaced growth increments lacking sharply defined boundaries, which were recogniz-
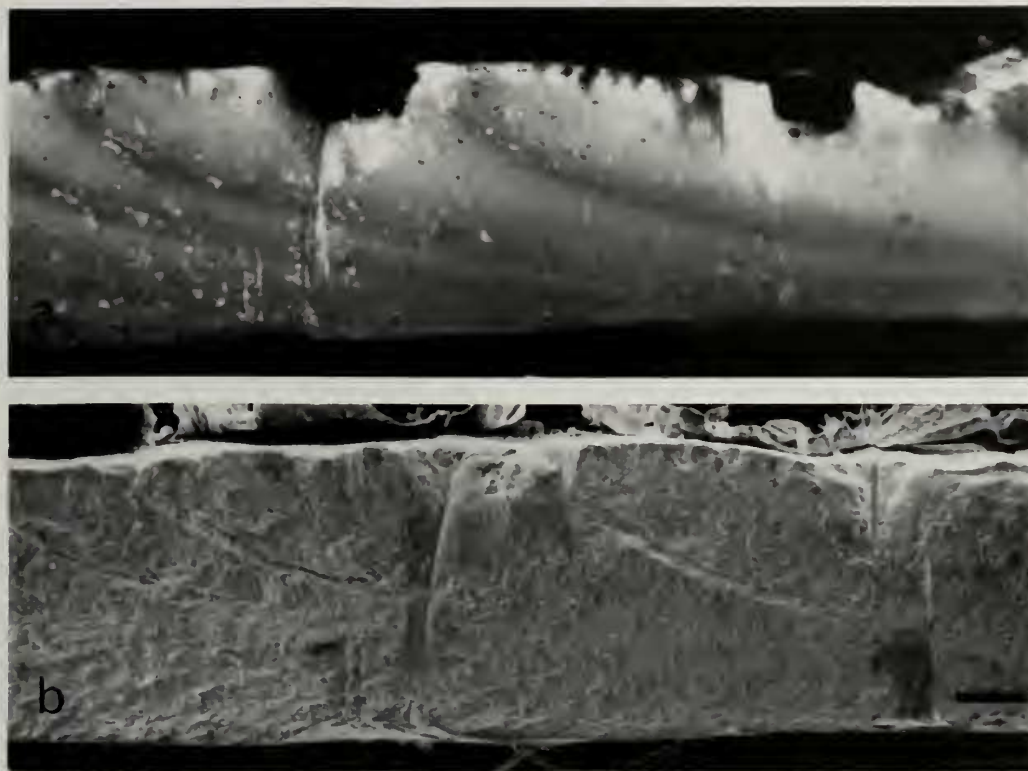
Figure 4

Radial, vertical shell sections of *Yoldia thraciaeformis* showing internal bands. Outer shell surface towards top of page. a. Photomicrograph showing an internal band bordered by two notch marks. b. Same section as viewed through SEM. Scale bar = 250 µm in both photographs.

able only through color variation. CLARK (1974) stated that the degree of exposure to environmental extremes can correlate well with growth line prominence. If we modify this to read that the magnitude of the causative biological or environmental variable can correlate with growth line prominence, then we see that whatever these factors are they can have as strong an influence in bivalves at 1000 m as in nearshore or intertidal bivalves.

Important to all environments, including the deep sea, is an understanding of environmental and biological processes in order to interpret growth increments. Unfortunately, seasonal data within Carson Canyon, at depths of 1000 m, are lacking. Measurements that have been taken at these depths elsewhere on the slope of the Newfoundland Grand Banks reveal temperatures in the 3–4°C range (CLARKE *et al.*, 1980). Limited seasonal data from the study region at depths of 500 m show almost constant temperature (3.5°C) and salinity (34.7 ppt) (Table 2). It is probable that there is little or no annual variation in temperature and salinity at slope depths of 1000–1500 m either; instead, minor variations (<1°C) occurring on a higher frequency basis (*e.g.*, 10-day period) probably exist (Clarke, personal communication). ELLETT & MARTIN

Table 1

Collection sites of *Nuculana pernula* and *Yoldia thraciae-formis* in Carson Canyon.

Date	Latitude	Longitude	Trawl depth (m)	Number of *N. per-nula*	Number of *Y. thra-ciae-formis*
3 June 1980	45°23′N	48°31′W	0895–0905	4	0
4 June 1980	45°18′N	48°35′W	1420–1500	32	85
4 June 1980	45°20′N	48°36′W	1020–1200	23	35
5 June 1980	45°18′N	48°33′W	1220–1280	101	20
8 June 1980	45°24′N	48°35′W	1000–1050	18	9
16 May 1981	45°33′N	48°10′W	1290–1320	1	0
16 May 1981	45°36′N	47°56′W	1460–1490	4	0
			Total	183	149

Table 2

Mean monthly temperature and salinity at 500 m over the Newfoundland continental slope (polygonal area delimited by 45°50′N, 47°56′W; 45°00′N, 48°56′W; 45°20′N, 48°56′W). Compiled data from stations (n) occupied over the period 1932 to 1983.[1]

		J	F	M	A	M	J	J	A	S	O	N	D	Overall
Temperature	$\bar{X}$	nd	nd	3.55	3.58	3.61	3.23	nd	3.33	nd	nd	nd	3.58	3.52
(°C)	SD			—	0.59	0.33	0.15		—				—	0.42
	n			1	9	8	4		1				1	24
Salinity	$\bar{X}$	nd	nd	34.74	34.79	34.67	34.74	nd	34.75	nd	nd	nd	34.46	34.72
(ppt)	SD			—	0.06	0.26	0.04		—				—	0.17
	n			1	9	8	3		1				1	23

nd = no data available.

1 = data courtesy of Marine Environmental Data Service, Department of Fisheries and Oceans, Ottawa, Canada.

(1973) recorded little or no variation in temperature and salinity throughout the year at depths of 2900 m in the Rockall Trough. However, at similar depths at this location, LIGHTFOOT *et al.* (1979) observed seasonal reproductive cycles in two deep-sea bivalve and brittlestar species. LIGHTFOOT *et al.* (1979) suggested that the timing of reproductive cycles in deep-sea bivalves and other deep-sea invertebrates indicated adaptive coupling to seasonal pulses in surface production. They cite evidence indicating that particulate organic material sinks to the deep sea rapidly enough that seasonal pulses in surface production are not completely damped out by differential sinking rates (BISHOP *et al.,* 1978; McCAVE, 1975; TURNER, 1977; VIEBE *et al.,* 1976; see also DICKSON *et al.,* 1982).

The distinctiveness of growth bands in the shells of *Yoldia thraciaeformis* and *Nuculana pernula* indicate that these deep-sea bivalves are strongly affected by an annual event throughout the lifetime of the organisms. Internal growth bands in these bivalves may represent reproductive cycle "markers" within the shell. Correlations have been made between timing of growth band formation and the reproductive cycle in shallow-water bivalves (JONES, 1980; PETERSON *et al.,* 1983); however, in these cases there is the compounding problem of significant annual water temperature changes, a factor absent from the deep-sea environment. Alternatively, growth bands in *Y. thraciaeformis* and *N. pernula* may represent shell-mediated changes in growth rate resulting from fluctuations in food supplied from surface waters.

ACKNOWLEDGMENTS

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